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(REV 5-93)

ATTORNEY'S DOCKET NUMBER: 24741-1523

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5): 09/807509

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/08678	11 November 1999	16 November 1998

TITLE OF INVENTION
PROCESS FOR PREPARING ANTIBODIES AGAINST A POLYPEPTIDE IN WHICH THE NUCLEIC ACID ENCODING THE PEPTIDE IS KNOWN (as amended)

APPLICANT(S) FOR DO/EO/US
Fritz GRUNERT, John THOMPSON, Wolfgang ZIMMERMANN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. _x_ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.

2. ___ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.

3. ___ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

4. _x_ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

5. _x_ A copy of the International Application as filed (35 U.S.C. 371©(2))

   a. _x_ is transmitted herewith (required only if not transmitted by the International Bureau).

   b. ___ has been transmitted by the International Bureau.

   c. ___ is not required, as the application was filed in the United States Receiving Office (RO/US)

6. _x_ A translation of the International Application into English (35 U.S.C. 371 ©(2)).

7. _x_ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371©(3))

   a. ___ are transmitted herewith (required only if not transmitted by the International Bureau).

   b. ___ have been transmitted by the International Bureau.

   c. ___ have not been made; however, the time limit for making such amendments has NOT expired.

   d. _x_ have not been made and will not be made.

8. ___ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371©(3)).

9. ___ An oath or declaration of the inventor(s) (35 U.S.C. 371©(4)).

10. _x_ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371©(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ___ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.

12. ___ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

13. _x_ A FIRST preliminary amendment.
    ___ A SECOND or SUBSEQUENT preliminary amendment.

14. ___ A substitute specification.

15. ___ A change of power of attorney and/or address letter.

16. _x_ Other items or information: **Notification of the Recording of a Change, Marked-up copy of amendments to claims, Unexecuted Declaration**

JC02 Rec'd PCT/PTO 16 APR 2001

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
-NEW 09/807509	PCT/EP99/08678	24741-1523

17. ☐ The following fees are submitted:

CALCULATIONS PTO USE ONLY

Basic National Fee (37 CFR 1.492(a)(1)-(5):
Neither international preliminary examination fee (CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....**$1000.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO . **$860.00**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....**$710.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4).....**$690.00**

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4)..... ..**$100.00**

ENTER APPROPRIATE BASIC FEE AMOUNT =				$860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than 20 ___ 30 ___ months from the earliest claimed priority date (37 CFR 1.492(e))				$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total Claims	18 -20 =	0	X $18.00	$	
Independent Claims	1-3 =	0	X $80.00	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)		0	+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				**$860.00**	
Processing fee of $130.00 for furnishing English translation later the 20 ___ 30 ___ months from the earliest claimed priority date (37 CFR 1.492(f).			+	$	
TOTAL NATIONAL FEE =				$860.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +				**$**	
TOTAL FEES ENCLOSED =				**$860.00**	
				Amount to be: refunded	$
				charged:	$

a. **X** A check in the amount of $860 to cover the above fees is enclosed.

b. ___ Please charge my Deposit Account No. 08-1641 in the amount of _____ to the above fees. A duplicate copy of this sheet is enclosed.

c. **X** The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 08-1641. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Patricia D. Granados April 16, 2001
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PATENT TRADEMARK OFFICE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of **Atty. Docket No. 24741-1523**

Fritz GRUNERT et al.

Serial No.: NEW

Filed: Herewith

For: PROCESS FOR PREPARING ANTIBODIES AGAINST A POLYPEPTIDE, THE NUCLEIC ACID ENCODING WHICH IS KNOWN

PRELIMINARY AMENDMENT

Director, U.S. Patent and Trademark Office
Washington, DC 20231

Sir:

Prior to examination, please amend the above-identified application as follows:

IN THE TITLE:

Please **amend the title** to read as follows:

--PROCESS FOR PREPARING ANTIBODIES AGAINST A POLYPEPTIDE IN WHICH THE NUCLEIC ACID ENCODING THE PEPTIDE IS KNOWN--

IN THE CLAIMS:

Please **amend claims 1-18** as follows:

1. (Amended) A process for producing antibodies which react specifically with a polypeptide, wherein the nucleic acid encoding the polypeptide is known, wherein:

(a) the DNA encoding the polypeptide is expressed in a host cell which is derived from a mammal using a vector which possesses at least one sequence encoding a detection

signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

(b) independently of step (a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide and the expression vector employed for the genetic immunization in step (b), for the purpose of preparing the desired antibodies, is also used *in vitro* for producing the target protein, and

(c) the antibodies which are formed in step (b) are reacted with the polypeptide formed in step (a) and detected or enriched.

2. (Amended) The process according to claim **1**, wherein the vector used in step (a) possesses, at the C-terminus of the DNA encoding the polypeptide, a sequence which encodes the detection signal.

3. (Amended) The process according to claim **2**, wherein the detection sequence is selected from the group consisting of His_6, tag sequence, the hemoglutinin sequence of an influenza virus and the myc tag sequence.

4. (Amended) The process according to claim 1, wherein the vector encoding the polypeptide posseses a polyadenylation sequence at the C-terminal end of the detection sequence.

5. (Amended) The process according to claim 1, wherein the vector encoding the polypeptide posseses a strong promoter at the 5′ end of the DNA sequence encoding the polypeptide.

6. (Amended) The process according to claim **5**, wherein the strong promoter is selected from the group consisting of strong eucaryotic promoters, in particular the elongation factor 1α promoter or the cytomegalovirus promoter.

7. (Amended) The process according to claim 1, wherein the polypeptide-encoding DNA which is introduced directly into an animal in accordance with step (b) is present in a vector.

8. (Amended) The process according to claim 1, wherein the polypeptide-encoding DNA is introduced into the animal in step (b) using a gene gun.

9. (Amended) The process according to claim 1, wherein the animal employed in step (b) is a mouse, a rat or a rabbit.

10. (Amended) The process according to claim 1, wherein in step (b), a genetic adjuvant is administered in addition to the polypeptide-encoding DNA.

11. (Amended) The process according to claim 10, wherein the genetic adjuvant is a cytokine expression vector which increases antibody production.

12. (Amended) The process according to claim 1, wherein suitable cells from an animal which has been immunized in accordance with step (b) are used for preparing hybridoma cells for forming monoclonal antibodies.

13. (Amended) The process according to claim 1, wherein the polypeptide formed in step (a) is bound to a solid phase by means of the detection signal being bound to an antibody or an antibody fragment which is directed against it.

14. (Amended) The process according to claim 13, wherein the solid phase is selected from the group consisting of microtiter plates, gel spheres and magnetic beads.

15. (Amended) The process according to claim 1, wherein the antibody formed in step (b) is detected, after having been bound to the polypeptide formed in step (a), using an anti-antibody which is detected against the antibody.

16. (Amended) The process according to claim 1, wherein the antibody which is reacted with the expressed polypeptide in step (c) is released by elution.

17. (Amended) The process according to claim 1, wherein the detection signal is a sequence which is responsible for membrane anchoring using a GPI residue.

18. (Amended) An antibody produced by a process comprising:

(a) expressing DNA encoding a polypeptide in a host cell which is derived from a mammal using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

(b) independently of step (a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide and the expression vector employed for the genetic immunization in step (b), for the purpose of preparing the desired antibodies, is also used *in vitro* for producing the target protein, and

(c) the antibodies which are formed in step (b) are reacted with the polypeptide formed in step (a) and detected or enriched.

REMARKS

Entry is respectfully requested of this Preliminary Amendment, the purpose of which is to place the claims in better form for examination in the U.S. Patent and Trademark Office. The above further amends the claims as amended on November 15, 2000, in PCT/EP99/08678.

Respectfully submitted,

April 16, 2001
Date

Patricia D. Granados
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Amendments to claims with brackets and underlining

Please **amend claims 1-18** as follows:

1. (Amended) **[**Process**]** A process for producing antibodies which react specifically with a polypeptide, wherein the nucleic acid encoding **[**which**]** the polypeptide is known, wherein:

(a) the DNA encoding the polypeptide is expressed in a host cell which is derived from a mammal using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

(b) independently of step (a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide and the expression vector employed for the genetic immunization in step (b), for the purpose of preparing the desired antibodies, is also used **[**in vitro**]** *in vitro* for producing the target protein, and

(c) the antibodies which are formed in step (b) are reacted with the polypeptide formed in step (a) and detected or enriched.

2. (Amended) **[**Process**]** The process according to claim **1**, **[**characterized in that**]** wherein the vector used in step (a) possesses, at the C-terminus of the DNA encoding the polypeptide, a sequence which encodes the detection signal.

3. (Amended) [Process] The process according to claim 2, [characterized in that] wherein the detection sequence is selected from the group consisting of His_6, tag sequence, the hemoglutinin sequence of an influenza virus [or] and the myc tag sequence.

4. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the vector encoding the polypeptide posseses a polyadenylation sequence at the C-terminal end of the detection sequence.

5. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the vector encoding the polypeptide posseses a strong promoter at the 5′ end of the DNA sequence encoding the polypeptide.

6. (Amended) [Process] The process according to claim 5, [characterized in that] wherein the strong promoter is selected from the group consisting of strong eucaryotic promoters, in particular the elongation factor 1α promoter or the cytomegalovirus promoter.

7. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the polypeptide-encoding DNA[,] which is introduced directly into an animal in accordance with step (b)[,] is present in a vector.

8. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the polypeptide-encoding DNA is introduced into the animal in step (b) using a gene gun.

9. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the animal employed in step (b) is a mouse, a rat or a rabbit.

10. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein in step (b), a genetic adjuvant is administered in addition to the polypeptide-encoding DNA.

11. (Amended) The process according to claim 10, [characterized in that] wherein the genetic adjuvant is [selected from a group comprising] a cytokine expression [vectors] vector which [increase] increases antibody production.

12. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein suitable cells from an animal which has been immunized in accordance with step (b) are used for preparing hybridoma cells for forming monoclonal antibodies.

13. (Amended) [Process] The process according to [one of the preceding claims, characterized in that] claim 1, wherein the polypeptide formed in step (a) is bound to a solid phase by means of the detection signal being bound to an antibody or an antibody fragment which is directed against it.

14. (Amended) [Process] The process according to claim 13, [characterized in that] wherein the solid phase is selected from the group consisting of microtiter plates, gel spheres [or] and magnetic beads.

15. (Amended) The process according to [one of the preceding claims, characterized in that] claim 1, wherein the antibody formed in step (b) is detected, after having been bound to the polypeptide formed in step (a), using an anti-antibody which is detected against the antibody.

16. (Amended) The process according to [one of the preceding claims, characterized in that] claim 1, wherein the antibody which is [bound] reacted with the expressed polypeptide in step (c) is released by elution.

17. (Amended) The process according to [one of the preceding claims, characterized in that] claim 1, wherein the detection signal is a sequence which is responsible for membrane anchoring using a GPI residue.

18. (Amended) **[**Antibody, characterized in that it can be obtained using one of the processes according to claims 1-17**]** An antibody produced by a process comprising:

(a) expression of DNA encoding a polypeptide in a host cell which is derived from a mammal using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

(b) independently of step (a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide and the expression vector employed for the genetic immunization in step (b), for the purpose of preparing the desired antibodies, is also used *in vitro* for producing the target protein, and

(c) the antibodies which are formed in step (b) are reacted with the polypeptide formed in step (a) and detected or enriched.

November 11, 1999

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Process for preparing antibodies against a polypeptide, the nucleic acid encoding which is known

Because of the enormous advances which have been made in the possibilities for sequencing nucleic acids, the problem frequently arises in molecular biology that, while the genetic information for a polypeptide or protein is known, this polypeptide or protein is not available in pure form. While nucleotide sequences are continually being published as a result of the Human Genome Project, the functions possessed by the polypeptides or proteins encoded by these genes are frequently completely unknown.

As a rule, it is very helpful for the practical application and evaluation of these scientific findings if these proteins can be detected using suitable antibodies. Such antibodies can be used either to purify the proteins or, for example, to determine the location of the proteins in tissues and cells.

An object of the present invention is therefore to make available antibodies which are directed against polypeptides or proteins, the nucleotide sequences for which are known but which are not available in enriched, and certainly not in purified, form.

Conventionally, antibodies are prepared by the proteins first of all being purified from the cells or the tissue, or being prepared recombinantly using bacteria, or in insect cells or mammalian cells, and these proteins being used for immunizing animals. These methods are frequently very elaborate and long-winded. The proteins which have been prepared in bacteria are frequently not identical to the naturally occurring

proteins since their secondary structure may differ from that of the native proteins and since bacteria do not possess the same post-translational modification mechanisms as those which are present in eucaryotic organisms.

The present invention therefore relates to a process for producing antibodies which react specifically with a polypeptide, the nucleic acid encoding which is known, in which process

a) the DNA encoding the polypeptide is expressed in a host cell using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

b) independently of step a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide, and

c) the antibodies which are formed in step b) are reacted with the polypeptide formed in step a) and detected or enriched.

The process according to the invention essentially consists of three steps. On the one hand, the DNA encoding the polypeptide is expressed in a suitable host cell using a vector (step a)). Since the polypeptide which is expressed using the vector is as a rule only present at relatively low concentration in the host cell, the vector employed is provided, according to the invention, with a nucleotide sequence which encodes a detection sequence (tag sequence). This tag sequence is linked to the sequence encoding the polypeptide, resulting in the expressed polypeptide possessing this detection peptide sequence at the C terminus, for example.

In step b), which is carried out independently of step a), the DNA encoding the polypeptide is introduced into a suitable animal and expressed in this animal. The genetic immunization which is employed in accordance with the invention enables antibodies to be formed directly in a host animal.

In this method of preparing antibodies, purified DNA, which contains the genetic information for the protein to be investigated and suitable control elements, is injected directly into the organism (mouse, rabbit, etc.) which is earmarked for the antibody production. The DNA is taken up by the cells of the recipient organism and the protein is expressed in native form (i.e. with correct post-translational modifications). The protein, which is foreign as far as the recipient organism is concerned, induces the immune system to produce antibodies which are directed against the foreign antigen (humoral immune response). This method has already been employed successfully for producing high-affinity monoclonal antibodies which recognise native proteins.

The expression vectors which are employed for the genetic immunization in step b), for the purpose of preparing the desired antibodies, are also to be used in vitro for producing the target protein. Transient transfection (electroporation, lipofection, etc.) is used to introduce the expression vectors into suitable target cells, in particular mammalian cells, which then synthesize the desired protein. These cells (intact or following lysis with suitable buffers) or medium supernatants (in the case of secreted proteins) are to be used for detecting the protein-recognizing antibody by means of FACScan analyses (in the case of proteins which are located in the cell) or ELISA.

When a foreign polypeptide is expressed in a host cell, the expressed polypeptide can usually be secreted to the exterior using a secretion sequence or leader sequence. In these cases, it is important that the expressed and secreted polypeptide possesses a

detection signal which can be used to isolate the polypeptide from the medium. If, however, the polypeptide is not secreted to the exterior but remains on the surface of the cell membrane, an additional detection sequence is then not absolutely necessary. In this case, the site in the polypeptide which is responsible for the anchoring between polypeptide and cell assumes the function of the detection sequence. Since, in this case, the expressed polypeptide remains linked to the cell, the antibodies which are formed can be detected by means of FACScan analyses, by binding to the polypeptide and subsequently reacting with a fluorescence-labeled antibody. As an alternative, it is also possible to carry out a cell ELISA in which the bound antibodies are detected using an enzyme-coupled secondary antibody and a suitable substrate reaction. If the anchoring sequence is a signal sequence which is responsible for anchoring to a membrane by way of a glycosylphosphatidylinositol (GPI) residue, the corresponding expression plasmid can then be used both for DNA immunization and for detecting the resulting antigen-specific antibodies, e.g. following transient transfection. The advantage of a GPI anchor is that it is easily cleaved enzymically from the cell surface in vivo and that it is consequently possible to achieve a good antibody reaction, as is known for secreted proteins (see Example 7 for a good immune response following genetic immunization with an expression plasmid which encodes a GPI-anchored protein).

In the case of secreted proteins (where appropriate, also in the case of proteins which are expressed intracellularly), it is necessary to attach a detection sequence (a tag) to the antigen recombinantly. This tag sequence enables the protein to be fished out of the cell supernatant or cell lysate using substances which interact with the tag sequence and which are bound to a solid matrix (e.g. antibodies which recognise the tag sequence; in the case of the His_6 tag sequence, suitable complexed Ni^{2+} ions).

Peptide sequences which are short and/or not very immunogenic are particularly suitable tag sequences. Mouse proteins which have a stimulatory effect on antibody production (e.g. GM-CSF, IL-4, IL-10, etc.) and which at the same time are able to function as tags can be used as tag sequences which are not particularly immunogenic (i.e. for preparing antibodies in mice). Such tags have the advantage of not developing any immune response because of the tolerance of the immunized animal towards these self-proteins. If it is not possible to prevent the formation of antibodies which recognise the tag sequence of a recombinant protein, these antibodies can be identified using constructs which encode irrelevant proteins which are provided with an identical tag.

The immobilized protein, which has been prepared by transient transfection, is now used to bind the antibodies, which recognise it, from the serum or the hybridoma culture supernatant (when preparing monoclonal antibodies). The bound, specific antibodies are then detected using enzyme-coupled anti-antibodies (detection antibodies) which are quantifiable, as a rule photometrically, by way of a specific substrate reaction. When using peptide tags, the specificity and sensitivity of the detection system can be significantly increased if $F(ab)_2$ fragments of the anti-tag antibody are used as captor antibodies and an Fc region-recognising antibody is used as the detection antibody. This configuration of the ELISA rules out any cross-recognition of the captor antibody.

The transcription unit which encodes the polypeptide can have a polyadenylation sequence, which is required for stabilizing a eucaryotic mRNA, at its 3' end.

In order to ensure that the polypeptide is expressed in the host cell, the vector normally possesses a promoter, with preference being given to using strong promoters. Examples which may be mentioned

are the elongation factor 1α promoter or the cytomegalovirus promoter.

In the process according to the invention, the nucleic acid encoding the polypeptide is introduced directly into an animal in order to produce antibodies against the polypeptide in this animal. In a preferred form, the DNA which is employed for this purpose is present in the form of a vector which is selected such that it can be used for the two steps a) and b) at one and the same time. In a particularly preferred embodiment, the polypeptide-encoding DNA is introduced using a so-called gene gun. In the gene gun method, microscopically small gold particles are coated with the DNA, preferably the vector or plasmid DNA, and shot at the shaved skin of the experimental animal. The gold particles then penetrate into the skin and express the DNA which has been applied to them in the host animal. Preference is given, according to the invention, to using laboratory animals such as mice, rats or rabbits.

In order to achieve a more vigorous antibody formation, so-called genetic adjuvants are, in a preferred embodiment, also applied simultaneously with the polypeptide-encoding DNA. These genetic adjuvants are plasmids which express cytokines (such as GM-CSF, IL-4 and IL-10) and which stimulate the humoral immune response in the laboratory animals.

Particularly when the laboratory animal employed is a mouse or a rat, there is the opportunity of forming hybridoma cells. The immunized mice are sacrificed, spleen cells are isolated and fused with tumor cells, and those clones which secrete the desired monoclonal antibodies are then selected.

In a particularly advantageous embodiment, the polypeptides to be investigated are secreted from the host cells in step a). Since a detection signal is linked to the polypeptides, the sought-after polypeptides can be isolated by forming a bond between the detection signal (tag sequence) and a suitable ligand. The tag sequence is preferably bound to a solid

phase. This solid phase can be the walls of microtiter plates, gel spheres or else magnetic beads. Magnetic beads have the advantage that the solution containing the expressed polypeptide can be readily mixed with the magnetic beads. The magnetic beads possess a ligand (for example antibody fragments) which binds to the tag sequence. The magnetic beads can then be concentrated by applying a magnetic field. By choosing suitable conditions, the sought-after polypeptide can then be eluted once again from the magnetic beads when the antibodies are to be enriched.

The present invention also relates to the antibodies which can be obtained using the process according to the invention.

Figure 1 shows the detection, by means of FACScan analysis, of anti-hp70 antibodies in serum and in the culture supernatant from hybridomas obtained from the lymph nodes of mice immunized with hp70-pcDNA3 DNA. BOSC cells which were either untransfected (gray curves) or transiently transfected with hp70-pcDNA3 DNA (white curves) were used for the FACScan analysis. GV114, mouse immunized with the hp70-pcDNA3 expression vector. The experiment is explained in more detail in example 7.

The present invention is explained in more detail with the aid of the following examples.

Example 1

Preparing murine monoclonal antibodies by means of genetic immunization without purified antigen (protein)

a) Expression construct for the genetic immunization

An expression construct based on the commercially available expression vector pcDNA3 (Invitrogen) was selected. In this vector, the cDNA is expressed under the control of the cytomegalovirus (CMV) promoter. However, it is also possible to use

other, preferably strong, usually ubiquitously active promoters (e.g. the promoter of the elongation factor 1α [EL-1α] gene). The human cDNA region encoding the extracellular domain of thyroid peroxidase (TPO) (2602 bp; 859 amino acids) was cloned into the BamHI/EcoRV cleavage sites in the polylinker sequence and additionally provided, at the 3' end, with a region encoding a His_6 tag and a subsequent stop codon: (TPO sol.-His-pcDNA3). The plasmid DNA was replicated in E. coli and purified using a Qiagen plasmid isolation kit (Qiagen, Hilden).

b) Genetic immunization of mice

In principle, there are two different methods for administering DNA for the genetic immunization. These methods are intramuscular injection or intracutaneous administration using gas pressure-accelerated, microscopically small gold particles coated with plasmid DNA (gene gun). We used the gene gun method for the Example. For this, 200 µg of TPO sol.-His-pcDNA3 DNA were applied per 25 mg of gold particles in accordance with the manufacturer's instructions (*gene gun optimization kit;* Bio-Rad, Munich). For the genetic immunization, the abdominal fur (approx. 4 cm^2) was removed, using perfume-free depilation cream (Veet), from five mice after they had been anaesthetized (intraperitonially) with 110 µl of ketamine/xylazine (100 mg/kg/16 mg/kg); the mice were then bombarded twice with the gene gun (Helios gene gun; Bio-Rad). 1 µg of plasmid DNA was administered per "bombardment". The immunization was repeated after 19 days, and blood was withdrawn 14 days later for determining the quantity of specific antibodies.

Example 2

Expressing the protein encoded by the expression construct

The protein encoded by the expression plasmid has to be prepared in order to detect the specific antibodies which are formed as a result of the genetic immunization. In order to obtain the protein in native form (as in the immunized animal), the expression construct was introduced by transfection into BOSC23 cells [Pear et al., (1993) PNAS, 84, 8392-8396]. BOSC23 cells are a modified adenovirus 5-transformed human embryonic kidney cell line (HEK293) which can be transiently transfected very satisfactorily. The cells were plated out in 6-well cell culture dishes such that they reached 80% confluence on the following day. They were then washed three times with in each case 2 ml of serum-free and antibiotic-free *Dulbecco's modified Eagle's medium* (DMEM) medium and treated with 2 µg of expression plasmid/10 µl of lipofectamine (Life Technologies, Eggenstein) in 1 ml of serum-free and antibiotic-free DMEM medium. The DNA/lipofectamine/medium mixture had previously been pipetted together in a polystyrene vessel and incubated for 10 minutes at room temperature. Following a 6-hour incubation at 37°C and 10% CO_2, 2 ml of DMEM/20% fetal calf serum (FCS) were added. 24 h after transfection (corresponds to the time at which the DNA was added), the medium was replaced with 5 ml of DMEM/5% FCS. After a further 48 h (72 h after transfection), the cell culture supernatant was removed and stored at -70°C.

Example 3

Detecting specific antibodies which are directed against the protein encoded by the expression construct

In order to bind the His_6 tag protein (TPO sol.-His) prepared by transient transfection to nickel chelate microtiter plates (DUNN, Asbach), the wells were in each case incubated, overnight at 4°C, with 200 µl of supernatant from the transient transfection mixture (see above) or of a mock-transfected BOSC23

culture supernatant and then washed 4 times with buffer A (50 mM tris/HCl, pH 7.5, 1 M NaCl) and twice with buffer B (phosphate-buffered saline (PBS), 0.1% BSA, 0.05% Tween 20). Nonspecific binding sites were then blocked by incubating with 300 µl of 3% bovine serum albumin (BSA)/PBS at room temperature for 1 h, after which the washes with buffer A and buffer B were repeated. The pre-immune sera and the immune sera from the immunized mice were diluted 1:100 with buffer B. In each case 100 µl of the diluted mouse sera were added to the wells of the nickel chelate microtiter plates. After incubating at room temperature for 1 hour, the wells were in each case washed four times with buffer C (50 mM tris/HCl, pH 7.5, 0.5 M NaCl, 0.1% BSA, 0.05% Tween 20) and twice with buffer B and then treated with 100 µl of rabbit anti-mouse Ig peroxidase conjugate (DAKO, Hamburg) diluted 1:2000 with buffer B. After a one-hour incubation, the wells were washed four times with buffer C and twice with buffer B and in each case treated with 100 µl of 3,3',5,5'-tetramethylbenzidene substrate solution (Fluka, Buchs, Switzerland). After sufficient development, the color reaction was stopped by adding 50 µl of 0.5 M H_2SO_4 and measured in an ELISA reader at a wavelength of 450 nm.

In order to check the serviceability of the invention which is presented here, the specific antibodies directed against TPO were detected "classically" by means of a commercially available TPO antibody ELISA (Varelisa TPO antibody; Pharmacia-Upjohn, Freiburg). In this test system, anti-TPO antibodies are detected using purified recombinant TPO. The content of anti-TPO antibodies in the pre-immune and immune sera from the immunized mice was determined at a dilution of 1:100 in accordance with the manufacturer's instructions.

Results:

It was possible to detect anti-TPO antibodies unambiguously, as compared with the pre-immune sera, at a dilution of 1:100, in the serum obtained from all the five mice which were immunized with TPO sol.-His-pcDNA3 DNA. The results are presented in Table 1.

Table 1: Detection of anti-TPO antibodies in the serum of TPO sol.-His-pcDNA3 DNA-immunized mice using purified TPO protein (*Varelisa TPO Antibodies* detection system).

Mouse	Optical density$_{450\ nm}$	
	Pre-immune serum	Immune serum
GV1	0.09	2.53
GV2	0.06	1.97
GV3	0.07	1.13
GV4	0.08	1.63
GV5	0.08	0.60

The detection system according to the invention was used to investigate the pre-immune serum and immune serum from a mouse (GV1 in Table 1) as an example. As can be seen from Table 2, it is possible to detect anti-TPO antibodies unambiguously, at a serum dilution of 1:100, in the immune serum whereas the pre-immune serum did not exhibit any reaction.

Table 2: Detection of anti-TPO antibodies in the serum of a TPO sol.-His-pcDNA3 DNA-immunized mouse using TPO sol.-His protein which was produced by transient expression.

Serum or buffer	Dilution with buffer A	Optical density	
		TPO sol.-His	Medium
pre-immune	1:100	0.17	0.15
immune	1:100	0.55	0.19

buffer A	--	0.03	0.01

Example 4

Preparing polyclonal antibodies by means of genetic immunization without purified antigen (protein) in rabbits

a) Expression construct for the genetic immunization

For the second case example, the ubiquitously active puomoter of the elongation factor 1α (EF-1α) gene was used for controlling the expression. The expression vector employed is based on the pBluescript vector (Stratagene, Heidelberg), into which a 1.2 kb fragment of the human EF-1α gene promoter, an 0.7 kb EcoRI fragment containing the polyadenylation signal from the cDNA for human G-CFS (Mizushima and Nagata, 1990), and also, between the BamHI and NotI cleavage sites, the oligonucleotide sequence encoding the influenza virus hemagglutinin (HA) tag were incorporated. The human cDNA region encoding the extracellular domain of the activin receptor IIA (431 bp; 135 amino acids) was cloned into the ClaI/BamHI cleavage sites of the polylinker sequence such that the HA tag-encoding region and a subsequent stop codon came to lie at the 3′ end (pEF-1α-ActRII-HA).

b) Genetic immunization of rabbits

For the genetic immunization, 100 μg of pEF-1α-ActRII-HA DNA were applied per 25 mg of gold particles in accordance with the manufacturer's instructions (*gene gun optimization kit;* Bio-Rad, Munich). After having been anaesthetized with 15 mg of pentobarbital/kg and having 200 cm^2 of the abdominal fur depilated with depilation cream, two rabbits (Chinchilla Bastard; Charles River, Sulzfeld) were

bombarded 30 times with the gene gun. 1 μg of plasmid DNA mixture was administered per "bombardment". The immunization was repeated after 21 days and blood was removed 21 days later for determining the quantity of specific antibodies.

Example 5

Expressing the protein encoded by the expression construct

The protein encoded by the expression plasmid pEF-1α-ActRII-HA was prepared, as described in Example 2, by transiently transfecting BOSC23 cells.

Example 6

Detecting specific antibodies which are directed against the protein encoded by the expression construct

In order to bind the HA tag protein (EF-1α-ActRII-HA), prepared by transient transfection, to microtiter plates, the wells were first of all coated with the $F(ab)_2$ fragment of the anti-HA tag antibody. For this, 150 μl of the antibody fragment were added to each well of the microtiter plate, after which the plate was washed with PBS at room temperature and free protein-binding sites were blocked by incubating with 200 μl of 0.2% BSA/PBS/well.

The supernatant from the transient transfection mixture (see Example 5), or from a mock transfected BOSC23 culture supernatant[sic], was then incubated at room temperature for 2 h, after which the plates were washed three times with phosphase-buffered saline (PBS). The pre-immune sera and immune sera from the immunized rabbits were diluted 1:100 and 1:500, respectively, with 0.2% BSA/PBS. 100 μl of the diluted rabbit sera were in each case added to the wells of the coated microtiter plates. After the plates had been

incubated at room temperature for one hour, the wells were in each case washed three times with PBS, after which 100 µl of goat anti-rabbit Ig peroxidase conjugate (DAKO, Hamburg), diluted 1:2000 with PBS/0.2% BSA) were added to each well. After the plate had been incubated for one hour, the wells were washed three times with PBS, after which 100 µl of 3,3',5,5'-tetramethylbenzidene substrate solution (Fluka, Buchs, Switzerland) were added to each well. After it had developed sufficiently, the color reaction was stopped by adding 50 µl of 0.5 M H_2SO_4 to each well and measured in an ELISA reader. The results showed that it is also possible to use the process according to the invention to produce specific polyclonal antibodies against an unknown gene product in rabbits.

Example 7

Using genetic immunization to prepare murine monoclonal antibodies against a human GPI-anchored surface protein

a) Expression construct for the genetic immunization

For the genetic immunization, the complete hp70 cDNA, encoding a 70 kDa GPI-anchored human surface protein, was cloned into pcDNA3 (hp70-pcDNA3) and replicated (see Example 1). Approx. 70% of the residues of the human hp70 amino acid sequence tally with those of the murine hp70 sequence.

b) Genetic immunization of mice

The mice were immunized with the gene gun (see Example 1b) using a short protocol (6 immunizations within 13 days), as described by Kilpatrick et al. (1998), Hybridoma 17: 569-576.

c) Preparing hybridomas for producing monoclonal antibodies

In order to prepare hybridomas, lymphocytes were isolated from the regional (axillary, brachial, inguinal and popliteal) lymph nodes from three mice and fused, in accordance with a standard protocol, with exponentially growing SP2/0 mouse myeloma cells (American Tissue Type Culture Collection) using polyethylene glycol (Sigma) (Campbell A M (1986). Monoclonal antibody technology: The production and characterization of rodent and human monoclonal antibodies. Book series: Laboratory Techniques in Biochemistry and Molecular Biology (R H Burdon and P H van Knippenberg, eds.), Elsevier Science Publishers, Amsterdam). 2 x 10^5 fused lymph node lymphocytes were plated out in each well of a 96-well microtiter plate and in each case cultured in 100 µl of hypoxanthine/aminopterin/thymidine (HAT)-containing DMEM medium (Sigma) containing 20% FCS and 5% *Hybridoma Enhancing Factor* (Sigma).

d) Detecting specific antibodies using cells in which the expression construct used for the genetic immunization is expressed following transient transfection

Candidate hybridoma clones were identified by means of a cell ELISA. For this, BOSC cells, as described in Example 2, were transiently transfected with the hp70-pcDNA3 expression construct, resuspended in 4% formaldehyde in PBS and fixed for 10 min. The cells were then diluted 1:10 with PBS and stored at 4°C for up to four weeks.

Cell ELISA

96-well round-bottom microtiter plates were blocked at room temperature by adding 300 µl of 1% BSA in PBS per well for 1 h. After the solution had been removed by inverting the plates, 75 µl of the hybridoma

cell supernatant and 10 µl of transiently transfected BOSC cell suspension (6 x 10^6 cells/ml of 1% BSA in PBS) were added per well and the plates were incubated at 4°C for 1 h. After 100 µl of 1% BSA in PBS had been added to the well, the plates were centrifuged at 300 x g for 4 minutes and the supernatants were tipped out as above. The cells were washed once again with 200 µl of 1% BSA/PBS/well, resuspended in each case in 75 µl of peroxidase-coupled goat anti-mouse immunoglobulin antibody (DAKO), diluted 1:2000 in 1% BSA/PBS, and incubated at 4°C for 1 h. 100 µl of 0.1% Tween 20/PBS were then added per well and the plates were centrifuged as above and the supernatants discarded. The cells were then washed three times with in each case 200 µl of 0.1% Tween 20/PBS and twice with in each case 200 µl of PBS. Peroxidase-coupled goat anti-mouse IgG antibody (diluted 1:2000) or goat anti-mouse IgM antibody (diluted 1:2000) (Southern Biotechnologies Associates) was used to determine the immunoglobulin class (IgG or IgM) of the monoclonal antibodies in the hybridoma supernatants. The peroxidase bound to the cells by way of the antibodies was quantified by adding 3,3',5,5'-tetramethylbenzidine substrate solution as described in Example 3.

Results:

In all, 176 hybridoma-covered microtiter wells were obtained using the above-described fusion. 64 supernatants from these wells proved to be positive for anti-hp70 antibodies when an OD_{450} value which was twice as high as the blank value obtained with medium (blank value: 0.035) was used as the threshold value. Table 3 lists the values which were measured for a negative (N1B10) hybridoma supernatant and for a positive (N1F4) hybridoma supernatant. The OD values obtained in the same test for the immune serum and pre-immune serum from a mouse (GV114) used for preparing the hp70 hybridoma are shown for comparison. The same N1B10 and

N1F4 hybridoma supernatants were also tested for the presence of specific anti-hp70 antibodies by means of a FACScan (*fluorescence-activated cell scanning*) analysis (see below).

Table 3: Use of a cell ELISA to detect anti-hp70 antibodies in serum and in culture supernatants from hybridomas obtained from the lymph nodes of mice immunized with hp70-pcDNA3 DNA. BOSC cells which were transiently transfected with hp70-pdDNA3 DNA were used for the cell ELISA.

Serum or hybridoma supernatant	Dilution	Optical density$_{450\ nm}$
Pre-immune serum GV114	1:100	0.08
Immune serum GV114	1:100	1.21
Hybridoma supernatant N1B10	undiluted	0.05
Hybridoma supernatant N1F4	undiluted	1.07

FACScan analysis

In each case 10 µl of the suspension of fixed, transiently transfected BOSC cells (20 x 10^6 in 3% FCS/PBS), as described under cell ELISA, were pipetted into the wells of a 96-well round-bottomed microtiter plate, after which 75 µl of the given hybridoma supernatant were added. As controls, cells were treated either with 25 µl of pre-immune or immune sera diluted 1:100 with 3% FCS/PBS or with 25 µl of a control monoclonal antibody (50 µg/ml of 3% FCS/PBS). After the plate had been incubated at 4°C for 30 min, 200 µl of 3% FCS/PBS were added to each well and the cells were centrifuged down, as described above, and the supernatants discarded. After the plate had been washed once with 200 µl of 3% FCS/PBS per well, 25 µl of a phycoerythrin-coupled goat anti-mouse immunoglobulin antibody (Southern Biotechnologies Associates), diluted 1:50 with 3% FCS/PBS (final concentration: 10 µg/ml),

were added per well and the plate was incubated at 4°C for 30 min. The cells were subsequently washed twice as above and the fluorescence was measured in a FACScan appliance (Becton Dickinson).

Results:

20 supernatants giving OD_{450} values of >0.2 were selected from the hybridoma supernatants judged to be positive in the cell ELISA (see above) for the determination of anti-hp70 antibodies by means of FACScan analysis. Figure 1B shows the histograms which were obtained, using BOSC cells which were transiently transfected with the hp70-pcDNA3 expression vector or which were not transfected, for an irrelevant antibody (26/3/13), used as negative control, and for the positive hybridoma supernatant N1F4. The histograms which were obtained in the same test for the immune and pre-immune sera from a mouse used for the hybridoma preparation are shown for comparison (Figure 1A). All the 20 hybridoma supernatants selected proved to be positive in the FACScan analysis. The immunoglobulin class of the hp70-specific antibodies was determined in 19 out of the total of 20 supernatants. Two of the tested supernatants contained hp70-specific IgM antibodies and 17 supernatants contained hp70-specific IgG antibodies.

Patent claims

1. Process for producing antibodies which react specifically with a polypeptide, the nucleic acid encoding which is known, wherein

a) the DNA encoding the polypeptide is expressed in a host cell which is derived from a mammal using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

b) independently of step a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide and the expression vector employed for the genetic immunization in step b), for the purpose of preparing the desired antibodies, is also used in vitro for producing the target protein, and

c) the antibodies which are formed in step b) are reacted with the polypeptide formed in step a) and detected or enriched.

2. Process according to claim 1, characterized in that the vector used in step a) possesses, at the C-terminus of the DNA encoding the polypeptide, a sequence which encodes the detection signal.

3. Process according to claim 2, characterized in that the detection sequence is selected from the His_6 tag sequence, the hemoglutinin sequence of an influenza virus or the myc tag sequence.

4. Process according to one of the preceding claims, characterized in that the vector encoding the polypeptide possesses a polyadenylation sequence at the C-terminal end of the detection sequence.

5. Process according to one of the preceding claims, characterized in that the vector encoding the polypeptide possesses a strong promoter at the 5' end of the DNA sequence encoding the polypeptide.

6. Process according to claim 5, characterized in that the strong promoter is selected from the group consisting of strong eucaryotic promoters, in particular the elongation factor 1α promoter or the cytomegalovirus promoter.

7. Process according to one of the preceding claims, characterized in that the polypeptide-encoding DNA, which is introduced directly into an animal in accordance with step b), is present in a vector.

8. Process according to one of the preceding claims, characterized in that the polypeptide-encoding DNA is introduced into the animal in step b) using a gene gun.

9. Process according to one of the preceding claims, characterized in that the animal employed in step b) is a mouse, a rat or a rabbit.

10. Process according to one of the preceding claims, characterized in that, in step b), a genetic adjuvant is administered in addition to the polypeptide-encoding DNA.

11. Process according to claim 10, characterized in that the genetic adjuvant is selected from a group comprising cytokine expression vectors which increase antibody production.

12. Process according to one of the preceding claims, characterized in that suitable cells from an animal which has been immunized in accordance with step b) are used for preparing hybridoma cells for forming monoclonal antibodies.

13. Process according to one of the preceding claims, characterized in that polypeptide formed in step a) is bound to a solid phase by means of the detection signal being bound to an antibody or an antibody fragment which is directed against it.

14. Process according to claim 13, characterized in that the solid phase is microtiter plates, gel spheres or magnetic beads.

15. Process according to one of the preceding claims, characterized in that the antibody formed in step b) is detected, after having been bound to the polypeptide formed in step a), using an anti-antibody which is directed against the antibody.

16. Process according to one of the preceding claims, characterized in that the antibody which is bound to the expressed polypeptide in step c) is released by elution.

17. Process according to one of the preceding claims, characterized in that the detection signal is a sequence which is responsible for membrane anchoring using a GPI residue.

18. Antibody, characterized in that it can be obtained using one of the processes according to claims 1-17.

Albert Ludwig University Freiburg

Abstract

A process is disclosed for producing antibodies which react specifically with a polypeptide, the nucleic acid encoding which is known, wherein

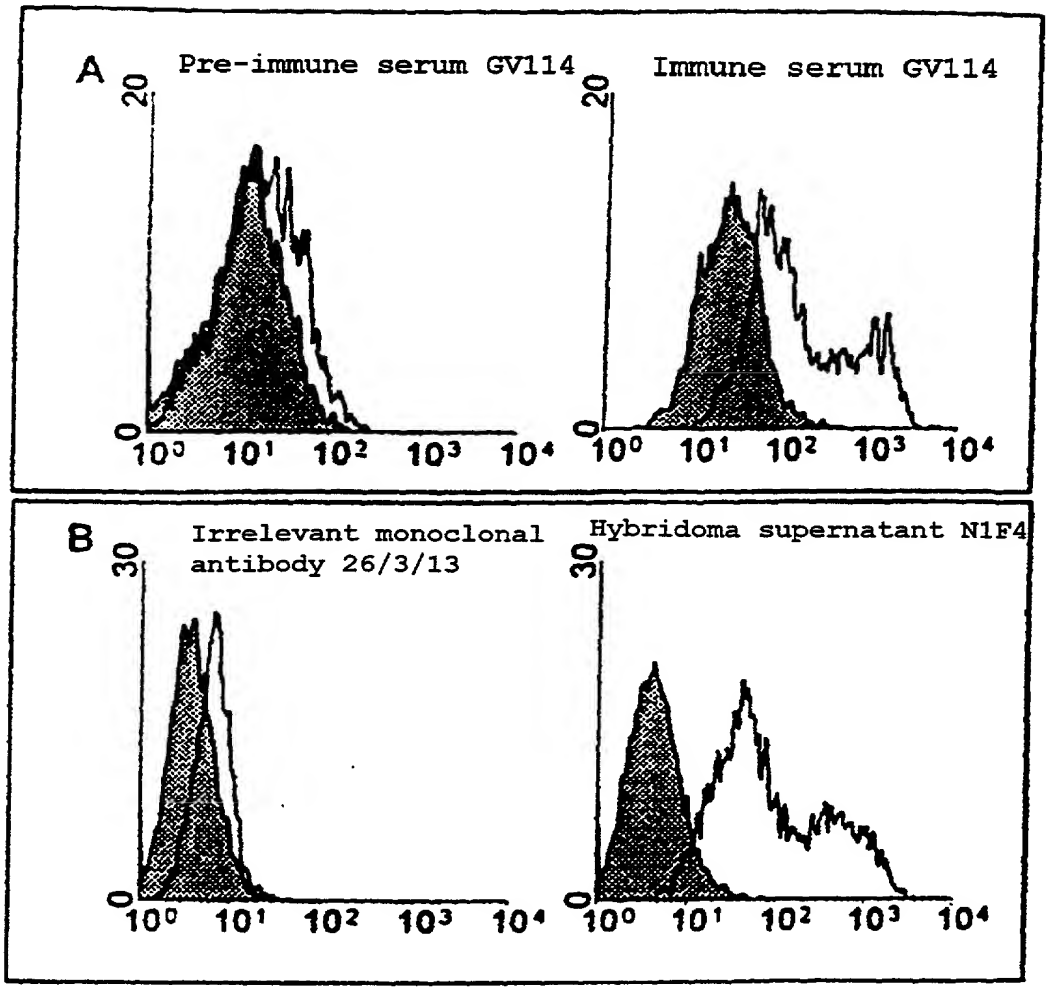
a) the DNA encoding the polypeptide is expressed in a host cell using a vector which possesses at least one sequence encoding a detection signal, and the expressed polypeptide is bound to a solid phase with the aid of the detection signal,

b) independently of step a), the DNA encoding the polypeptide is introduced directly into an animal, resulting in expression of a polypeptide in the animal, which expression causes the formation of antibodies against the polypeptide, and

c) the antibodies which are formed in step b) are reacted with the polypeptide formed in step a) and detected or enriched.

09/807509

Fig. 1



Attorney Docket No. 24741-1523

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I HEREBY DECLARE:

THAT my residence, post office address, and citizenship are as stated below next to my name:

THAT I believe I am the original, first, and sole inventor (if only one inventor is named below) or an original, first, and joint inventor (if plural inventors are named below or in an attached Declaration) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROCESS FOR PREPARING ANTIBODIES AGAINST A POLYPEPTIDE IN WHICH THE NUCLEIC ACID ENCODING THE PEPTIDE IS KNOWN.

the specification of which (check one)

___ is attached hereto.
X was filed as United States Application No. or **PCT International Application No. PCT/EP99/08678** and was amended on **November 15, 2000** (if applicable).

THAT I do not know and do not believe that the same invention was ever known or used by others in the United States of America, or was patented or described in any printed publication in any country, before I (we) invented it;

THAT I do not know and do not believe that the same invention was patented or described in any printed publication in any country, or in public use or on sale in the United States of America, for more than one year prior to the filing date of this United States application;

THAT I do not know and do not believe was first patented or made the subject of an inventor's certificate that issued in any country foreign to the United States of America before the filing date of this United States application if the foreign application was filed by me (us), or by my (our) legal representatives or assigns, more than twelve months (six months for design patents) prior to the filing date of this United States application;

THAT I have reviewed and understand that contents of the above-identified specification, including the claim(s), as amended by any amendment specifically referred to above;

THAT I believe that the above-identified specification contains a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention, and sets forth the best mode contemplated by me of carrying out the invention; and

THAT I acknowledge the duty to disclose to the U.S. Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I HEREBY CLAIM foreign priority benefits under Title 35, United States Code §119(a)-(d) of §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number	Country	Foreign Filing Date	Priority Claim?	Certified Copy Attached?
198 52 800.0	FED. REP. GERMANY	16 November 1998	Yes	No

I HEREBY CLAIM the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

U.S. Provisional Application Number	Filing Date

I HEREBY CLAIM the benefit under Title 35, United States Code, §120 of any United States application(s), or §365(c) of any PCT International application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

U.S. Patent Application Number	PCT Patent Application Number	Patent Filing Date	Parent Patent Number

I HEREBY APPOINT the following registered attorneys and agents of the law firm of Heller Ehrman White & McAuliffe LLP to have full power to prosecute this application and any continuations, divisions, reissues, and reexaminations thereof, to receive the patent, and to transact all business in the United States Patent and Trademark Office connected therewith:

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I UNDERSTAND AND AGREE THAT the foregoing attorneys and agents appointed by me to prosecute this application do not personally represent me or my legal interests, but instead represent the interests of the legal owner(s) of the invention described in this application.

I FURTHER DECLARE THAT all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so make are punishable by a find or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-00

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Atty. Docket No: 24741-1523

In re patent application of
GRUNERT, Fritz *et al.*

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Filed: June 25, 2001 Examiner: Unassigned

For: PROCESS FOR PREPARING ANTIBODIES AGAINST A POLYPEPTIDE IN WHICH THE NUCLEIC ACID ENCODING THE PEPTIDE IS KNOWN

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Sir:

Applicant respectfully requests that the official correspondence address for the above captioned application be changed to Patricia D. Granados and the attorneys of Heller Ehrman White & McAuliffe LLP, located at 1666 K Street, N.W., Suite 300, Washington, D.C. 20006. Please direct all written communications to Patricia D. Granados at the above mentioned address, and direct all telephone communications to (202) 912-2000.

Respectfully submitted,

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